Establishing human skin tissue on Nunc Cell Culture Inserts in Carrier Plate Systems

Abstract

Thermo Scientific™ Nunc™ Cell Culture Inserts provide an excellent cell growth system for growing tissues that require an air–liquid interface, especially when used in conjunction with their corresponding carrier plate. The carrier plate has multiple positions to place the inserts to facilitate the handling of the inserts and reduce contamination risk. These multiple positions can be used to increase the volume of medium used for culturing, which can extend the interval between required changes of the medium during tissue growth. Here we examine the effectiveness of the Nunc Cell Culture Inserts in Carrier Plates for producing 3D epidermal skin models.

Key words

Epidermal, 3D tissue, human skin model, cell culture inserts, carrier plate, air–liquid interface, feeding interval, TEER

Introduction

Artificially grown skin models have become an important substitute for actual skin to simulate the effects of different conditions (e.g., acute toxicity, allergenicity, inflammation) on epidermal tissues. Inducing keratinocytes to differentiate into the various layers of epidermis requires direct exposure to air as well as to the culture medium that supplies the nutrients for cell growth and differentiation. This type of air–liquid interface culture can be accomplished by growing cells on a porous membrane carefully positioned in the culture well, allowing the upper surface of the cells to be exposed to the air, while the lower surface of the cells is fed and wetted by medium through the pores of the growth membrane.

The Nunc cell culture insert is a porous membrane device that is commonly used for small-scale 3D skin growth. Cells are seeded in the cell culture insert, and the insert is submerged in medium in a multi-well plate. Once the cell layer is established on the insert's porous membrane, the medium above the membrane is removed, exposing the upper surface of the cells to the air.

The main drawback of this type of system is the limited space below the growth membrane that only allows a small volume of medium in each well to sustain cell growth during the air–liquid interface culture. This necessitates a short feeding cycle during the prolonged culture period required for cell differentiation and expansion. Performing medium changes with cell culture inserts can be a tedious task, as the inserts sit loose in the wells and must be worked around or removed during pipetting steps, increasing the chances of contaminating and damaging the 3D model or the porous membrane. The Nunc Carrier Plate System addresses these issues by suspending the cell culture inserts above the wells at the desired height. The carrier plate allows a greater volume of medium to be used in the wells during the air–liquid interface culture, potentially extending the time intervals between medium changes. If needed, the carrier plate can be removed from the wells to facilitate aseptic maneuvering, taking all of the installed inserts with it and allowing easy access for aspirating and pipetting the medium from the entire plate. The lid covering the carrier plate keeps the inserts and the cells protected from potential contaminants.

In this study, we established an effective system for culturing 3D skin epidermal tissue *in vitro* using the Nunc Cell Culture Inserts with Gibco™ EpiLife™ growth medium. We also used the adjustable-height capabilities of the carrier plate to simplify the experimental protocol, extending feeding intervals and saving time and labor.

Materials and methods

Materials	Cat. No.
Nunc Cell Culture Inserts in Carrier Plate Systems, 24-well, 0.4 µm pore size	141002
Human Epidermal Keratinocytes, adult (HEKa)	C-005-5C
EpiLife Medium, with 60 µM calcium	M-EPI-500-CA
Human Keratinocyte Growth Supplement (HKGS)	S-001-5
Antibiotic-Antimycotic (100X)	15240-062
Coating Matrix Kit Protein	R-011-K
FGF7 (KGF) Recombinant Human Protein	PHG0094
CyQUANT MTT Cell Proliferation Assay Kit	V13154
Ascorbic Acid	A4544-25G

Air–liquid interface culture on inserts

EpiLife growth medium was prepared by adding HKGS, 10 ng/mL KGF, 1X antibiotic-antimycotic solution, and 140 µM calcium chloride. Prior to using the medium, aliquots were supplemented with 50 µg/mL ascorbic acid. Cell culture inserts were precoated with a 1:100 dilution of the protein from the Coating Matrix Kit, according to the manufacturer's protocol.

For the initial cell attachment and expansion, all inserts were set in the lowest position of the carrier plate in their respective wells. Cells were seeded in precoated inserts at a density of 750,000 cells/cm^2. The culture area of the 24-well insert is 0.47 cm^2. Cells were seeded with 0.5 mL of growth medium in the lower compartment and 0.5 mL of cell suspension in the upper compartment. After 2 days of incubation at 37°C and 5% CO_2, the air–liquid interface was established by aspirating all of the medium from the wells and from inside of the cell culture insert, then adding the appropriate volume of growth medium to the lower compartment and repositioning the inserts at the desired hanging height in the 24-well plate (Table 1). The upper compartment, inside of the cell culture insert, was left empty. Subsequent medium changes were conducted by aspirating the medium from the lower compartment and replacing it with fresh medium supplemented with an additional 1.5 mM calcium chloride (1.7 mM total) at the desired interval (Table 1).

Table 1. Choose the insert hanging position and medium volume according to your desired interval for changing the medium.

Insert-hanging position	Volume of growth medium for lower compartment in a 24-well plate*	Interval for changing medium
Low	0.5 mL/well	2 days
Middle	1.0 mL/well	3 days
High	1.5 mL/well	4 days

* The volume for a 12-well carrier plate is twice that of the 24-well carrier plate.

Viability assay

Cell viability and metabolism were assessed after 23 days in culture using the Invitrogen™ CyQUANT™ MTT Cell Proliferation Assay Kit. The MTT reagent was added into the upper compartment of the inserts and incubated for 1 hour. The MTT solution was then aspirated, and the cells were washed. The formazan dye was extracted from the cell layer overnight using 100% isopropyl alcohol. The extracts were then transferred to individual wells in a clear 96-well plate and tested for absorbance using a Thermo Scientific™ Varioskan™ Flash plate reader. Viability was assessed in 6 inserts.

Trans-epithelial electrical resistance (TEER) measurement

The TEER was measured at 2 time points during the growth of the skin tissue—at 11 and 23 days post-seeding. Measurements were taken using an EVOM2™ Epithelial Volt/Ohm (TEER) Meter and probe (World Precision Instruments). During the recording, the cell growth medium was aspirated and replaced with 0.5 mL phosphate-buffered saline (PBS) in both the upper and lower compartments. The probe was placed such that one electrode was submerged in the upper compartment and the other was submerged in the lower compartment. TEER values were recorded for 6 inserts at each time point.

Microscopic examination

Skin tissue in inserts was allowed to grow for 12 days post-seeding and then fixed using an overnight incubation in 4% paraformaldehyde at 4°C. Inserts were paraffin-embedded and sectioned followed by processing for hematoxylin and eosin (H&E) staining. Tissue sections were photographed at 400x magnification to examine the stratification of cell layers.

Results

Initial experiments of cell attachment and expansion indicated that the Nunc cell culture inserts were an excellent growth substrate for human epidermal keratinocytes. An MTT assay after 2 weeks of air–liquid interface culture showed good viability in all wells tested (Figure 1).

TEER was used to determine the strength of the barrier formed by the skin tissue established in the inserts. A mature layer of skin tissue should prevent the flow of ions across the porous growth membrane, as indicated by a high level of electrical resistance. Our measurements taken on culture days 11 and 23 showed high levels of resistance, indicating a strong barrier formed as early as day 11 and continuing to mature to day 23, 3 weeks after air exposure (Figure 2).

Following 12 days of air–liquid interface culture, examination of fixed and sectioned artificial skin tissue by microscope indicated good differentiation of the epidermal layers. All of the expected cell types were visible in the sectioned and stained tissues (Figure 3).

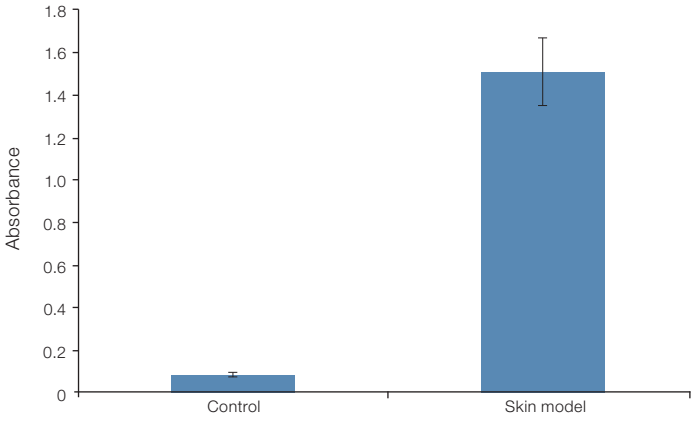

Figure 1. Assessment of cell viability by MTT assay. Mean absorbance of extracts from skin model samples compared to control inserts without cells. Error bars indicate standard deviation.

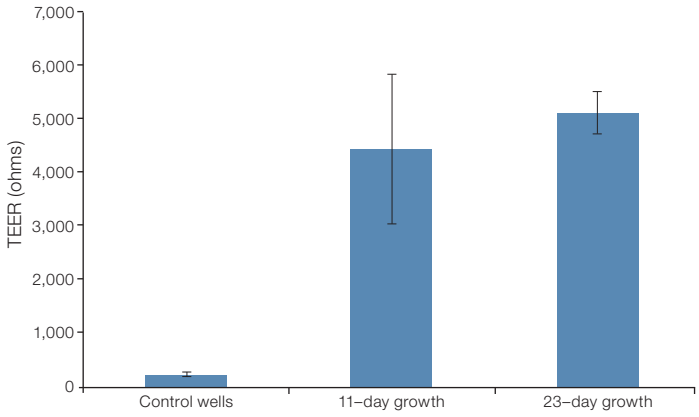

Figure 2. Assessment of skin model integrity using TEER measurement. TEER measurements were taken at 11 and 23 days after the initial seeding of the cells in the inserts. TEER values were compared to control inserts of the same size without cells. Error bars indicate standard deviation.

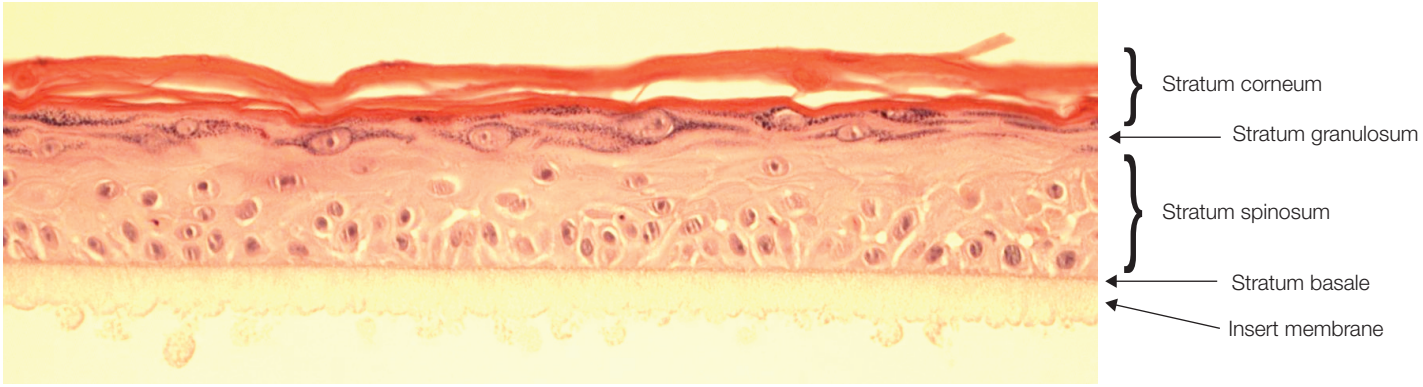

Figure 3. Stratification of the epidermal skin model on the insert membrane was shown by H&E staining.

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Additional experiments were performed to determine whether feeding intervals had a significant impact on epidermal tissue formation in the cell culture inserts. We took advantage of the versatility of the carrier plates and tested different insert-hanging positions, and the corresponding increases in media volume for different media change intervals of 2, 3, or 4 days (Figure 4). For all intervals tested, the resulting skin tissue showed good differentiation of the cell layers after 12 days of air–liquid interface culture, indicating that the longer intervals worked as effectively as the shorter ones, and saved time and labor during establishment of skin tissue (Figure 5).

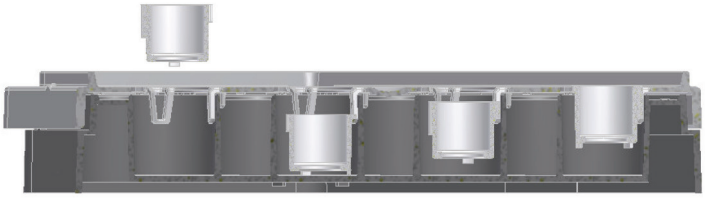

Figure 4. Cross-sectional view of the Nunc Carrier Plate with 3 hanging positions for the Nunc Cell Culture Inserts.

Conclusion

- The Nunc Cell Culture Inserts in Carrier Plate Systems provide an excellent and convenient method for culturing artificial models of human skin tissue.
- The multiple height settings of the carrier plate allow for increased volumes of growth medium during air–liquid interface culture, extending the medium change intervals for more convenient laboratory procedures.

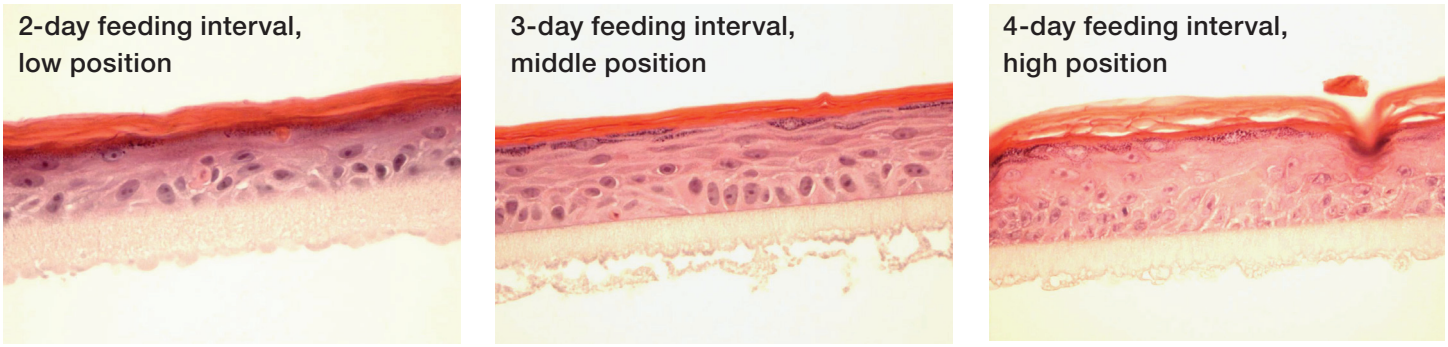

Figure 5. H&E staining of stratification of the epidermal skin model after 12 days of air–liquid interface culture with media changes at varying intervals. Different volumes of growth medium were used with different insert-hanging positions in the carrier plate to achieve the air–liquid interface culture. Epidermal tissue was cultured in 0.5 mL, 1.0 mL, or 1.5 mL medium per well and fed every 2, 3, or 4 days, respectively. Tissue sections were photographed at 400x magnification.

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